

CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

- Claim 1 (withdrawn):** A method for characterizing a promoter comprising:
providing a construct comprising said promoter operably linked to a nucleic acid encoding a cytoplasmic form of chitobiase;
introducing the construct into host cells; and
identifying sequences in said promoter which regulate transcription levels.
- Claim 2 (withdrawn):** The method of Claim 1, wherein said cytoplasmic form of chitobiase lacks a signal sequence.
- Claim 3 (withdrawn):** The method of Claim 2, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.
- Claim 4 (withdrawn):** The method of Claim 1, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. 0-7*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium discoideum*, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*, *Mus musculus*, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.
- Claim 5 (withdrawn):** The method of Claim 1, wherein said method of identifying sequences which are involved in directing transcription comprises mutagenizing said promoter.
- Claim 6 (withdrawn):** The method of Claim 1, wherein said method of identifying sequences which are involved in transcription comprises constructing deletions in said promoter.

Claim 7 (original): A method for identifying a regulatory element capable of directing or regulating transcription within a test nucleic acid sequence comprising:

providing a construct comprising said test nucleic acid sequence operably linked to a nucleic acid encoding a cytoplasmic form of chitobiase;
introducing said construct into host cells; and
determining the level of chitobiase activity.

Claim 8 (original): The method of Claim 7, wherein said cytoplasmic form of chitobiase lacks a signal sequence.

Claim 9 (original): The method of Claim 8, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.

Claim 10 (original): The method of Claim 7, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. 0-7*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium discoideum*, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*, *Mus musculus*, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.

Claim 11 (original): The method of Claim 7, wherein said reporter gene construct is introduced transiently.

Claim 12 (original): The method of Claim 7, wherein said reporter gene construct is introduced stably.

Claim 13 (original): The method of Claim 7, wherein said host cells are selected from the group consisting of prokaryotic cells and eukaryotic cells.

Claim 14 (original): The method of Claim 7, further comprising permeabilizing or lysing said host cells.

Claim 15 (original): The method of Claim 14, wherein said permeabilizing or lysing step comprises treating said host cells with toluene.

Claim 16 (original): The method of Claim 7, wherein said step of determining the level of chitobiase activity is selected from the group consisting of measuring the amount of a chemiluminescent product produced from a substrate, measuring the amount of a fluorescent product produced from a substrate, measuring the amount of light absorbed by a product produced from a substrate and measuring a decrease in the amount of a detectable substrate.

Claim 17 (currently amended): The method of Claim 7, wherein said step of determining the level of ~~chitobiase~~ chitobiase activity comprises determining the level of *p*-nitrophenol released from a substrate.

Claim 18 (original): The method of Claim 7, wherein said test nucleic acid sequence comprises a portion of genomic DNA.

Claim 19 (original): The method of Claim 7, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after exposing said host cells to a desired set of environmental conditions.

Claim 20 (currently amended): The method of Claim 7, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after contacting said host cells with a compound to be tested for its influence on the level of ~~transcription~~ transcription from ~~said regulatory~~ said regulatory element.

Claim 21 (withdrawn): A method of detecting successful transformation, comprising the steps of:

introducing a nucleic acid encoding a cytoplasmic form of chitobiase into host cells; and
detecting chitobiase expression in said host cells.

Claim 22 (withdrawn): A fusion protein-reporter gene construct comprising a promoter operably linked to a nucleic acid encoding a cytoplasmic form of chitobiase fused in frame with a nucleic acid encoding a heterologous polypeptide, wherein said heterologous polypeptide is not β -galactosidase or a portion thereof and wherein said heterologous polypeptide does not contain a signal peptide.

Claim 23 (withdrawn): The nucleic acid of Claim 22, wherein said nucleic acid encodes a cytoplasmic form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. 0-7*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium discoideum*, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*, *Mus*

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musculus, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.

Claim 24 (withdrawn): The nucleic acid of Claim 22, further comprising a λ site-specific recombination sequence.

Claim 25 (withdrawn): A reporter gene construct comprising plasmid pJMF3.

Claim 26 (withdrawn): A reporter gene construct comprising plasmid pJMF4.

Claim 27 (withdrawn): A reporter gene construct comprising plasmid pDYK9.

Claim 28 (withdrawn): A reporter gene construct comprising plasmid pDYK11.

Claim 29 (withdrawn): A host cell comprising the construct of Claim 22.

Claim 30 (withdrawn): The host cell of Claim 29 wherein said nucleic acid is integrated into a chromosome of said cell.

Claim 31 (withdrawn): The host cell of Claim 29, wherein said nucleic acid is transiently expressed in said host cell.

Claim 32 (withdrawn): A nucleic acid encoding a cytoplasmic form of chitobiase in which the signal sequence of native chitobiase has been inactivated or deleted.

Claim 33 (withdrawn): The nucleic acid of Claim 32, wherein the signal sequence has been mutated to inactivate it.

Claim 34 (withdrawn): An isolated or purified polypeptide comprising a cytoplasmic form of chitobiase fused in frame with a heterologous polypeptide, wherein said heterologous polypeptide is not β -galactosidase or a portion thereof and wherein said heterologous polypeptide does not contain a signal peptide.

Claim 35 (withdrawn): An isolated or purified polypeptide comprising a cytoplasmic form of chitobiase in which the signal peptide of native chitobiase has been inactivated or deleted.

Claim 36 (withdrawn): The polypeptide of Claim 35, wherein the signal sequence has been mutated to inactivate it.

Claim 37 (withdrawn): A method for monitoring the activity of a promoter comprising:
providing a construct comprising said promoter operably linked to a nucleic acid encoding a cytoplasmic form of chitobiase;
introducing said construct into host cells; and

determining the level of chitobiase activity.

Claim 38 (withdrawn): The method of Claim 37, wherein said cytoplasmic form of chitobiase lacks a signal sequence.

Claim 39 (withdrawn): The method of Claim 38, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.

Claim 40 (withdrawn): The method of Claim 37, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. 0-7*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium discoideum*, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*, *Mus musculus*, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.

Claim 41 (withdrawn): The method of Claim 37, wherein said reporter gene construct is introduced transiently.

Claim 42 (withdrawn): The method of Claim 37, wherein said reporter gene construct is introduced stably.

Claim 43 (withdrawn): The method of Claim 37, wherein said host cells are selected from the group consisting of prokaryotic cells and eukaryotic cells.

Claim 44 (withdrawn): The method of Claim 37, further comprising permeabilizing or lysing said host cells.

Claim 45 (withdrawn): The method of Claim 44, wherein said permeabilizing or lysing step comprises treating said host cells with toluene.

Claim 46 (withdrawn): The method of Claim 37, wherein the step of determining the level of chitobiase activity is selected from the group consisting of measuring the amount of a chemiluminescent product produced from a substrate, measuring the amount of a fluorescent product produced from a substrate, measuring the amount of light absorbed by a product produced from a substrate and measuring a decrease in the amount of a detectable substrate.

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Claim 47 (withdrawn): The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of *p*-nitrophenol released from a substrate.

Claim 48 (withdrawn): The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after exposing said host cells to a desired set of environmental conditions.

Claim 49 (withdrawn): The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after contacting said host cells with a compound to be tested for its influence on the level of transription from siad regularatory element.

Claim 50 (withdrawn): The method of Claim 49, wherein said compound comprises a compound to be tested for activity as a drug.